



The
Patent
Office

GB99/4386

PCT/GB 99 / 04376



INVESTOR IN PEOPLE

PRIORITY DOCUMENT

SUBMITTED OR TRANSMITTED IN
COMPLIANCE WITH RULE 17.1(a) OR (b)

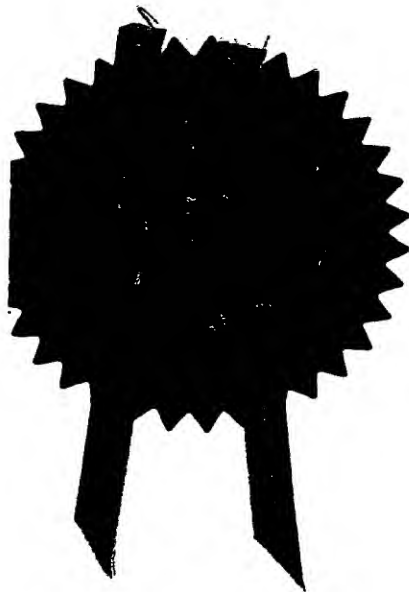
The Patent Office
Concept House
Cardiff Road
Newport
South Wales
NP10 8QQ

I, the undersigned, being an officer duly authorised in accordance with Section 74(1) and (4) of the Deregulation & Contracting Out Act 1994, to sign and issue certificates on behalf of the Comptroller-General, hereby certify that annexed hereto is a true copy of the documents as originally filed in connection with the patent application identified therein.

In accordance with the Patents (Companies Re-registration) Rules 1982, if a company named in this certificate and any accompanying documents has re-registered under the Companies Act 1980 with the same name as that with which it was registered immediately before re-registration save for the substitution as, or inclusion as, the last part of the name of the words "public limited company" or their equivalents in Welsh, references to the name of the company in this certificate and any accompanying documents shall be treated as references to the name with which it is so re-registered.

In accordance with the rules, the words "public limited company" may be replaced by p.l.c., plc, P.L.C. or PLC.

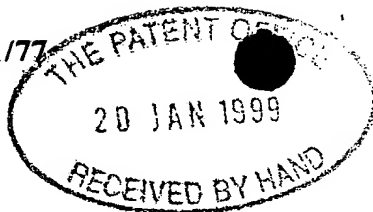
Re-registration under the Companies Act does not constitute a new legal entity but merely subjects the company to certain additional company law rules.



L. Mahoney

Signed

Dated 14 January 2000



The
**Patent
Office**

1/77

21 JAN 99 F419356-8 D02890

P01/7700 0.00 - 9901233.8

The Patent Office

Request for the grant of a patent

(See the notes on the back of this form. You can also get an explanatory leaflet from the Patent Office to help you fill in this form)

Cardiff Road
Newport
Gwent NP9 1RH

1. Your reference

REP06007GB

2. Patent a/
(The Patent)

9901233.8

20 JAN 1999

3. Full name, address and postcode of the or of each applicant (underline all surnames)

Microscience Limited
67-68 Jermyn Street
London
SW1Y 6NY
United Kingdom

Patents ADP number (if you know it)

If the applicant is a corporate body, give the country/state of its incorporation

United Kingdom

7583685001

4. Title of the invention

PROTEIN AND COMPOSITIONS
CONTAINING IT

5. Name of your agent (if you have one)

GILL JENNINGS & EVERY

"Address for service" in the United Kingdom to which all correspondence should be sent (including the postcode)

Broadgate House
7 Eldon Street
London
EC2M 7LH

Patents ADP number (if you know it)

745002

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and (if you know it) the or each application number

Country

Priority application number
(if you know it)

Date of filing
(day / month / year)

7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

Number of earlier application

Date of filing
(day / month / year)

8. Is a statement of inventorship and of right to grant of a patent required in support of this request? (Answer 'Yes' if:

YES

- a) any applicant named in part 3 is not an inventor
 - b) there is an inventor who is not named as an applicant, or
 - c) any named applicant is a corporate body.
- See note (d))

• **Patents Form 1/77**

9. Enter the number of sheets for any of the following items you are filing with this form.
Do not count copies of the same document

Continuation sheets of this form

Description	8
Claim(s)	1
Abstract	
Drawing(s)	3

10. If you are also filing any of the following, state how many against each item.

Priority documents

Translations of priority documents

Statement of inventorship and right to grant of a patent (*Patents Form 7/77*)

Request for preliminary examination and search (*Patents Form 9/77*)

Request for substantive examination
(*Patents Form 10/77*)

Any other documents
(*please specify*)

11. For the Applicant
Gill Jennings & Every

I/We request the grant of a patent on the basis of this application.

Signature

Date

20 January 1999

12. Name and daytime telephone number of person to contact in the United Kingdom

PERRY, Robert Edward
0171 377 1377

Warning

After an application for a patent has been filed, the Comptroller of the Patent Office will consider whether publication or communication of the invention should be prohibited or restricted under Section 22 of the Patents Act 1977. You will be informed if it is necessary to prohibit or restrict your invention in this way. Furthermore, if you live in the United Kingdom, Section 23 of the Patents Act 1977 stops you from applying for a patent abroad without first getting written permission from the Patent Office unless an application has been filed at least 6 weeks beforehand in the United Kingdom for a patent for the same invention and either no direction prohibiting publication or communication has been given, or any such direction has been revoked.

Notes

- If you need help to fill in this form or you have any questions, please contact the Patent Office on 0645 500505.
- Write your answers in capital letters using black ink or you may type them.
- If there is not enough space for all the relevant details on any part of this form, please continue on a separate sheet of paper and write "see continuation sheet" in the relevant part(s). Any continuation sheet should be attached to this form.
- If you have answered 'Yes' Patents Form 7/77 will need to be filed.
- Once you have filled in the form you must remember to sign and date it.
- For details of the fee and ways to pay please contact the Patent Office.

PROTEIN AND COMPOSITIONS CONTAINING IT

Field of the Invention

This invention relates to one protein, to vaccines
5 containing it, and to its use in therapy, for immunisation.

Background to the Invention

Group B Streptococcus (GBS), also known as
Streptococcus agalactiae, is the causative agent of various
conditions. In particular, GBS causes:

10 *Early onset neonatal infection.*

This infection usually begins in utero and causes
severe septicaemia and pneumonia in infants, which is
lethal if untreated and even with treatment is associated
with a 10-20% mortality rate.

15 *Late onset neonatal infection.*

This infection occurs in the period shortly after
birth until about 3 months of age. It causes a
septicaemia, which is complicated by meningitis in 90% of
cases. Other focal infections also occur including
20 osteomyelitis, septic arthritis, abscesses and
endophthalmitis.

Adult infections.

These appear to be increasingly common and occur most
frequently in women who have just delivered a baby, the
25 elderly and the immunocompromised. They are characterised
by septicaemia and focal infections including
osteomyelitis, septic arthritis, abscesses and
endophthalmitis.

Urinary tract infections.

30 GBS is a cause of urinary tract infections and in
pregnancy accounts for about 10% of all infections.

Veterinary infections.

GBS causes chronic mastitis in cows. This, in turn, leads to reduced milk production and is therefore of considerable economic importance.

5 GBS infections can be treated with antibiotics. However, immunisation is preferable. It is therefore desirable to develop an immunogen that could be used in a therapeutically-effective vaccine.

Summary of the Invention

10 According to the present invention, a partial GBS gene sequence for the protein Phosphoglycerate Kinase (PGK) has been found which represents an outer surface protein.

15 In one aspect of the invention, the use of this protein in a recombinant protein vaccine is described. This vaccine may be administered to females either prior to, or during pregnancy to protect mother and neonate against infection by GBS.

20 The gene sequence may be first genetically altered to increase the antigenicity of the encoded protein.

Brief Description of the Drawings

The invention will now be described in detail with reference to the accompanying figures, wherein:

25 Figure 1a shows the 5'-terminal partial nucleotide sequence and the deduced N-terminal amino acid sequence of the GBS PGK.

Figure 1b shows the 3'-terminal partial nucleotide sequence and the deduced C-terminal amino acid sequence of the GBS PGK.

30 Figure 2a shows peptide sequence of the PGK obtained from MS/MS peptide sequencing

Figure 2b shows the sequence of oligonucleotides derived from the sequences in 2a used for PCR amplification of GBS PGK.

Description of the Invention

Because of its extracellular or cell surface location, the protein of the present invention may be a
5 suitable candidate for the production of therapeutically-
effective vaccines against GBS. The term
"therapeutically-effective" is intended to include the
prophylactic effect of the vaccines. For example, a
10 recombinant protein may be used, as an antigen for direct
administration to an individual. The protein may be
isolated directly from GBS or expressed in any suitable
expression system, e.g. *Lactococcus lactis*. It is
preferably administered with an adjuvant, e.g. alum.

The protein may be a mutant protein in comparison to
15 wild-type protein, a fragment of the protein or a
combination of different fragments, provided an effective
immune response is generated.

An alternative approach is to use a live attenuated
GBS vaccine. This may be produced by deleting the gene
20 that encodes the protein. Preferably, the GBS strain
comprises additional virulence gene mutations.

The protein (or fragments thereof) of the present
invention may also be used to produce monoclonal and
polyclonal antibodies for use in passive immunisation.

25 In a further embodiment of the invention, the
protein or corresponding polynucleotide may be used as a
target for screening potentially useful drugs, especially
antimicrobials. Suitable drugs may be selected for their
ability to bind to the protein to exert their effects.
30 Assays for screening for suitable drugs and which make
use of the protein of the invention will be apparent to
those skilled in the art.

Although the protein has been described for use in
the treatment of individuals, veterinary uses of the

protein are also considered to be within the scope of present invention. In particular, the protein or the vaccines may be used in the treatment of chronic mastitis, especially in cows.

5 The present invention is described with reference to Group B Streptococcal strain M732. However, all the GBS strains and many other bacterial strains are likely to include related proteins having amino acid sequence homology with the protein of M732. Organisms likely to
10 contain the proteins include, but are not limited to, *S. pneumoniae*, *S. pyogenes*, *S. suis*, *S. milleri*, Group C and Group G *Streptococci* and *Enterococci*. Vaccines to each of these may be developed in the same way as described for GBS.

15 Preferably, the proteins that may be useful for the production of vaccines have greater than 40% sequence similarity with the protein of M732. More preferably, the proteins have greater than 60% sequence similarity. Most preferably, the proteins have greater than 80%
20 sequence similarity.

 The protein of the present invention was identified as follows:

 Todd-Hewitt Broth was inoculated with GBS and allowed to grow overnight at 37°C. The cells were
25 harvested by centrifugation and washed with Phosphate Buffered Saline (PBS). The cells were resuspended in an osmotic buffer (20%(w/v) Sucrose, 20mM Tris-HCl pH 7.0, 10mM MgCl₂) containing protease inhibitors (1 mM PMSF, 10
30 μ M Iodoacetic Acid, 10 mM 1,10-Phenanthroline, 1 μ M Pepstatin A) and Mutanolysin at a final concentration of 4 Units per microlitre. This was incubated (shaking) at 37°C for 2 hours.

 Cells and debris were removed first by high speed centrifugation, then ultra-centrifugation for 1

hour. The resultant supernatant containing cell wall proteins was concentrated under pressure using an ultrafiltration device (10,000 molecular weight cut-off).

The sample was dialysed against ultra high
5 quality water and lyophilised. After resuspension in loading buffer, the proteins were separated by preparative 2-Dimensional-Gel Electrophoresis. Following Electrophoresis an individual spot was chosen for study. The spot was subjected to in-gel tryptic digestion. The
10 resulting peptides were extracted from the gel and purified using microbore RP-HPLC. Fractions were collected every 45 seconds and a portion of these consistent with the regions of UV absorbance were analysed by Delayed Extraction-Matrix Assisted Laser
15 Desorbition-Time of Flight Mass Spectrometry (DE-MALDI-TOF-MS). Peptides not observed in a blank preparation were then subjected to sequencing using Nanospray-MS/MS

The Peptide Sequences obtained are shown in Figure 2a.

20 Using this information, degenerate oligonucleotides were designed to be used in a polymerase chain reaction (PCR) to amplify the DNA segment lying between the peptide sequences identified. The sequences of these oligonucleotides is shown in Figure 2b.

25 PCR amplification resulted in the production of an (approximately) 800 base pair fragment, which was cloned into the pCR 2.1-TOPO vector (Invitrogen BV, Netherlands) according to manufacturers protocol. This plasmid was termed pMS11. The cloned DNA fragment was sequenced.
30 Sequence obtained from the 5' and 3' ends of the cloned fragment were used to design primers for genomic sequencing of the upstream and downstream region of the original sequence. In total approximately 1150 nucleotides have now been sequenced, including a defined

5'-translation initiation start signal and a termination signal. (Figure 1a and 1b) The deduced amino acid sequence from both fragments was used to search protein databases. Results of this search are shown in
5 Table 1 and 2.

As shown in Table 1 and 2, homologues to the GBS MS11 gene product can be identified in *Lactobacillus delbrueckii*, *Thermotoga maritima*, *Clostridium acetobutylicum*, *Bacillus megaterium*, *Triticum aestivum*
10 and *Synechocystis PCC6803*.

In all cases the homologues are the genes for the protein Phosphoglycerate Kinase (PGK). PGK is a major enzyme in the glycolytic pathway, being involved in the conversion of Glyceraldehyde-3-phosphate to
15 Phosphoenolpyruvate. In particular, it is involved in the catalysis of the reaction between Glycerate-1,3-diphosphate and 3-Phospho-Glycerate, releasing a phosphate in the forward reaction.

Table 1. Database search results for MS11 N-terminal

Organism	Protein Accession	DNA Accession	Gene Name	% Similari ty	% Identity	Alignment Length
<i>Lactobacillus delbrueckii</i>	O32756	AJ000339	Phosphoglycerate Kinase	78	63	124
<i>Thermotoga maritima</i>	P36204	X75437	Phosphoglycerate Kinase	80	64	70
<i>Clostridium acetobutylicum</i>	O52632	AF043386	Phosphoglycerate Kinase	83	67	62
<i>Bacillus megaterium</i>	P24269	X54519	Phosphoglycerate Kinase	78	58	70

Table 2. Database search results for MS11 C-terminal

Organism	Protein Accession	DNA Accession	Gene Name	% Similari ty	% Identity	Alignment Length
<i>Lactobacillus delbrueckii</i>	O32756	AJ000339	Phosphoglycerate Kinase	72	57	112
<i>Clostridium acetobutylicum</i>	O52632	AF043386	Phosphoglycerate Kinase	57	43	102
<i>Triticum aestivum</i>	P12783	X15232	Phosphoglycerate Kinase	60	45	85
<i>Synechocystis PCC6803</i>	P74421	2499503	Phosphoglycerate Kinase	61	45	73

CLAIMS

1. A protein comprising an amino acid sequence encoded by the polynucleotide defined as MS11 in Figure 1a or 1b, or a homologue thereof with at least 60% sequence homology.
2. A protein according to claim 1, obtainable from the Group B streptococcal strain M732.
3. A protein according to claim 1 or claim 2, wherein MS11 comprises the nucleotides 1-600 of Figure 1a and/or nucleotides 1-550 of Figure 1b.
4. A protein according to any of claims 1 to 3, for use in a method of therapy.
5. A polynucleotide which encodes a protein according to any preceding claim, its complement, or a fragment thereof.
6. The use of a bacterial protein according to any of claims 1 to 4, in the manufacture of a vaccine to treat bacterial infection.
7. The use according to claim 6, wherein the infection is a Group B streptococcal infection.
8. The use according to claim 6 or claim 7, wherein the infection is a focal infection.
9. The use according to claim 6 or claim 7, wherein the infection is a urinary tract infection.
10. Use of a product according to any of claims 1 to 5, for screening potential antimicrobial drugs.
11. An antimicrobial drug selected using the products as defined in claim 10.
12. A vaccine comprising a product according to any of claims 1 to 5.
13. A vaccine comprising a microorganism having a virulence gene deletion, wherein the gene codes for a protein according to any of claims 1 to 4.
14. An antibody raised against a protein according to any of claims 1 to 4.



Figure 1a. 5' Nucleotide and deduced N-terminal amino acid sequence of clone MS11

```

      10                               30                               50
ATGGCTAAATTGACTGTAAAGACGTTGATTTGAAGGTAAAAAAGTCCT
M A K L T V K D V D L K V K K V L

      60                               80                               100
CGTTCGTGTTGACTTTAATGTGCCTTTGAAAGACGGCGTTATCACTAACG
V R V D F N V P L K D G V I T N D

      110                              130                              150
ACAACCGTATCACTGCGGCTCTTCCAACAATCAAGTATATCATCGAACAA
N R Y T A A L P T I K Y I I E Q

      160                              180                              200
GGTGGTCGTGCTATCCTCTTCTCTCACCTTGGACGTGTTAAAGAAGAAGC
G G R A I L F S H L G R V K E E A

      210                              230                              250
TGACAAAGAAGGAAAATCACTTGCACCGGTAGCTGCTGATTTAGCTGCTA
D K E G K S L A P V A A D L A A K

      260                              280                              300
AACTTGGTCAAGATGTTGTATTCCCAGGTGTTACTCGTGGTGCAAATTA
L G Q D V V F P G V T R G A K L

      310                              330                              350
GAAGAAGCAATCAATGCTTTGGAAGATGGACAAGTTCTTTTGGTTGAAA
E E A I N A L E A G Q V L L V E N

      360                              380                              400
CACTCGTTTTTGAAGATGTTGACGGTAAGAAAGAATCTAAGAATGACGAAG
T R F E D V D G K K E S K N D E E

      410                              430                              450
AACTTGGTAAATACTGGGCTTCACTTGGAGATGGAATCTTCGTTAACGAT
L G K Y W A S L G D G I F V N D

      460                              480                              500
GCATTTGGTACAGCACACCGTGCTCATGCATCAAACGTAGGTATTTTCAGC
A F G T A H R A H E S N V G I S A

      510                              530                              550
AAACGTTGAAAAGCTGTAGCTGGTTTCTTCTTGAAAACGAAATTRCTT
N V E K A V A G F L L E N G I A Y

      560                              580                              600
ACATCCAAGAAGCAGTTGAAACTCCAGAACGCCAATTCGTAGCTATCTTG
I Q E A V E T P E R Q F V A I L
```



.....

Figure 1b. 3' Nucleotide and deduced C-terminal amino acid sequence of clone MS11

```

10                               30                               50
□
AAAGCTGATAAAGTTCTTATCGGTGGTGGTATGACTTACACATTCTACAA
 K A D K V L I G G G M T Y T F Y K

70                               90                               100
AGCTCAAGGTATCGAAATCGGTAACCTCACTTGTAGAAGAAGACAAATTGG
□
 A Q G I E I G N S L V E E D K L D

110                              130                              150
ATGTTGCTAAAGACCTCCTTGAAAAATCAAACGGTAAATTGATCTTGCCA
 V A K D L L E K S N G K L I L P

170                              190                              200
GTTGACTCAAAAGAAGCAAACGCATTTGCTGGTTATACTGAAGTTCGCGA
 V D S K E A N A F A G Y T E V R D

210                              230                              250
CACTGAAGGTGAAGCAGTTTCAGAAGGGTTCCTTGGTCTTGACATCGGTC
 T E G E A V S E G F L G L D I G P

270                              290                              300
CTAAATCAATCGCTAAATTTGATGAAGCACTTACTGGTGCTAAACAGTT
 K S I A K F D E A L T G A K T V

310                              330                              350
GTATGGAACGGACCTATGGGTGTCTTTGAAAACCCCTGACTTCCAAGCTGG
 V W N G P M G V F E N P D F Q A G

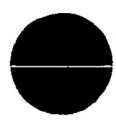
370                              390                              400
TACAATCGGTGTAATGGACGCTATCGTTAAACAACCAGGCGTTAAATCAA
 T I G V M D A I V K Q P G V K S I

410                              430                              450
TCATCGGTGGTGGTGATTTCAGCAGCAGCTGCTATCAACCTTGGTCGTGCT
 I G G G D S A A A A I N L G R A

470                              490                              500
GACAAATTCTCATGGATCTCTACTGGTGGTGGAGCAAGCATGGAATTGCT
 D K F S W I S T G G G A S M E L L

510                              530                              550
CGAAGGTAAAGTATTACCAGGTTTGGCAGCATTGACTGAAAAATAA
 E G K V L P G L A A L T E K *

```



C



Figure 2a. Generated Peptide Sequences for MS11

(L/I)GQDVVF

(L/I)GGGDSAAA

Figure 2b. Oligonucleotide sequences designed from Peptide sequences in Figure 2a

GGWCAAGATGTWGTWTT

WGCWGCWGCWSWATCWCCWCCWCC

PCT/213 99/04246

22-12-99

Full Jennings - Every